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TITLE: Cellular Mechanisms Regulating uPA in Hormone Refractory Prostate Cancer: A Novel Therapeutic Target

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13. ABSTRACT *(Maximum 200 Words)*

DOD grant DAMD17-00-1-0524 has the purpose of inhibiting the expression of two pathways critical to prostate cancer progression, one mediated by the tyrosine kinase receptor c-Met, and the second mediated by urokinase plasminogen activator (uPA). Dr. Gary E. Gallick, Ph.D. became PI of the project upon after the departure from M.D. Anderson of Dr. Robert Radinsky. Because of the requirement for approval of change of PI and use of animals, work on this proposal commenced after January 1, 2001. Since that time, work has progressed on task 2, which is to directly test the role of HGF/c-Met and uPA on the invasion and metastasis of human prostate tumor cells. To accomplish this task, PC3 sublines have been developed which express a c-Met ribozyme (to lower c-Met expression) and a control ribozyme. These cell lines are being screened currently for altered c-Met expression. The effects of the uPA inhibitor A5 are being compared with those of A6, a new and potentially more potent inhibitor of this pathway.

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INTRODUCTION

DOD Award number DAMD17-00-1-0524, "Cellular Mechanisms Regulating Urokinase-Type Plasminogen Activator in Hormone Refractory Prostate Cancer: A Novel Therapeutic Target" has its goal inhibiting the expression of two pathways critical to prostate cancer progression, one mediated by the tyrosine kinase receptor c-Met, and the second mediated by urokinase plasminogen activator (uPA). Each pathway may be important to prostate tumor progression, and further, they may be related, i.e., increased c-Met expression leads directly to increased uPa expression. The purpose of the research is to provide a novel therapeutic basis for the development of prostate tumor metastases through inhibition of these growth regulatory pathways. DAMD17-00-1-0524 was originally awarded to Robert Radinsky, Ph.D. with the award date of July 1, 2000. Dr. Radinsky subsequently left the University of Texas M.D. Anderson Cancer Center for Amgen Pharmaceuticals and I (Gary E. Gallick, Ph.D.) was approved to become the Principal Investigator of this grant. In the past four months since work on the project has begun, we have focused on the development of cell lines with inhibited c-Met expression, as proposed in Task 2, as theses cell lines are absolutely required to test the overall hypothesis that c-Met expression is important to metastatic development of prostate cancer. Progress on this task is described in this report.

BODY

As indicated in the introduction, I (Gary E. Gallick, Ph.D), was approved to become PI of this work after the departure of Robert Radinsky, Ph,D., from The M.D. Anderson Cancer Center. Before the full award could be made, the U.S. Army veterinarians raised several concerns with respect to the procedures in the proposed studies. I therefore undertook a thorough review of the proposal, with emphasis on the studies requiring animals, and determined that modifications of protocols needed to be written and approved by our Institutional Use and Care of Animals Committee (IUCAC) prior to initiation of the studies. These modifications were written and received IUCAC approval prior to responding to the concerns of the U.S. Army veterinarians. Approval was received from the Army veterinarians for use of animals. As a result of these necessary delays, no money was expended on this grant prior to January, 2001, and work on the project did not begin until this time. After approval, supplies were ordered, cell lines left by Dr. Radinsky were thawed, such that the proposed experiments could be completed. As a result, this progress report represents work on the project for approximately only four months.

Development of Prostate tumor cell lines with reduced c-Met expression

The success of this work depends, in large part, upon generating clones of prostate tumor cell lines in which c-Met expression is reduced. Therefore, we have chosen to focus our efforts first on task 2, the isolation of PC3-LN4 clones with reduced c-Met expression through the transfection of a c-Met ribozyme. The first element in that task was the construction of expression vectors for ribozymes directed against c-Met mRNA. While the proposal described strategies to obtain

hammerhead ribozymes, the laboratory of John Laterra demonstrated success in this approach (Abounader et al., 1990. Prior to Dr. Radinsky leaving the Institution, he received the ribozyme-encoding plasmid from Dr. Laterra. Therefore, it was unnecessary for us to construct a ribozyme-expressing plasmid from the beginning.

The next part of task 2 was to isolate PC3-LN-4 cells with reduced c-Met expression. To accomplish this task, we have transfected the plasmid encoding the ribozyme into the cells, and selected for G418 resistance by standard techniques. This is the strategy in the original proposal. We have been successful in obtaining G418 resistant colonies, and have expanded them into stable cell lines. Currently, we have isolated 17 G418-resistant clones with active ribozyme, and 10 with an inactive ribozyme control. We are in the process of characterizing these clones in terms of c-Met expression and in vitro growth as described in the proposal. Thus, in the upcoming year, we expect to be able to complete the next tasks outlined in the proposal, confirm reduced c-Met expression and evaluate clones for in vitro growth and tumorigenicity and metastasis.

While this strategy may be successful, a potential problem in completing task 2 is the stability of clones expressing the ribozyme constructs. This is of some concern, as collaborative studies with Dr. Radinsky in colon cancer (not related to DOD funding) had revealed that expression of the ribozyme may be lost after continued passage. To overcome this potential problem, Dr. Laterra at Johns Hopkins has developed an Adenovirus that expresses c-Met ribozymes and an Adenovirus that expresses a ribozyme to Hepatocyte Growth Factor/Scatter Factor (HGF/SF), the ligand for c-Met. We have just completed an Institutional Materials and Transfer Agreement to obtain these Adenoviruses from Dr. Laterra, and written an animal protocol for their potential use. This strategy of inhibiting c-Met is an attractive alternative to the plasmid-driven reduction of c-Met expression, as it allows greater reduction in expression of c-Met. Importantly, use of this strategy does not change in any way the tasks of the proposal, but facilitates the inhibition of c-Met by more recent technologic advances. The minor change in strategy was discussed with Dr. Mishra Nrusingha, Grants Manager, Congressionally Directed Medical Research Programs, U.S. Army Medical Research and Materiel Command, who concurred that this approach does not change the Statement of Work.

Inhibition of c-Met-mediated signaling by PTEN/MMAC

We fully anticipate one of the two strategies above to prove successful for inhibiting HGF/SF-mediated pathways. However, PTEN/MMAC, a lipid phosphatase and tumor suppressor gene inactivated frequently in prostate cancer (Li et al., 1997; Steck et al., 1997) has been implicated in inhibiting some of the same pathways. In work not funded by the DOD, we have recently examined the effects of ectopic expression of MMAC/PTEN on the growth of PC3 cells in an orthotopic model for prostate cancer, and preliminary studies demonstrate that MMAC/PTEN expression strongly inhibits the development of lymph node metastases. Whether this inhibition is due directly to inhibition of HGF and uPA-mediated pathways will be determined in other studies in the laboratory. Should the same pathways be affected, we wish to propose the use of MMAC/PTEN expressing PC3 cells as another model to inhibit the pathways studied by this grant. As this strategy is not outlined in the original Statement of Work, permission of the Grants Officer would be sought before pursuing this line of investigation.

uPA inhibitors

An additional requirement of Task 2 is the inhibition of the urokinase Plasminogen Activator Receptor (uPAR) pathway by inhibiting the binding of urokinase plasminogen activator (uPA) through the use of A5, a novel and potent uPA antagonist. We have initiated studies of inhibition of this pathway in prostate tumor cells in collaboration with Douglas Boyd, Ph.D., Department of Cancer Biology, M.D. Anderson Cancer Center. Recently, a more potent inhibitor, termed A6 has been developed. We have obtained this inhibitor and are comparing its ability to antagonize the uPA binding to uPAR. Once these studies are completed, we will then utilize either A5 or A6 in the studies of prostate cells, using exactly the strategy in the original proposal.

Tasks 1 and 3

Task 1 involves the evaluation of the ability of high and low metastatic prostate cancer cells to invade a three dimensional matrix after HGF treatment in the presence of uPA antagonists. Part of this task will be performed on cell lines with reduced c-Met expression isolated above. Therefore, work on this task will commence in year 2. Task 3 involves examination of surgical specimens. This task was not proposed to begin until year 2, and will be delayed somewhat due to the late start of the work.

KEY RESEARCH ACCOMPLISHMENTS

- Development of 17 PC3 cell lines expressing the c-Met ribozyme
- Development of 10 cell lines expressing a control (inactive) ribozyme
- Inhibition of uPAR pathway with the specific A5 and A6 inhibitors
- Inhibition of development of PC3 metastases by ectopic expression of MMAC/PTEN, a downstream regulator of c-Met-mediated signal transduction

REPORTABLE OUTCOMES

-Development of PC3 subclones with expression of c-Met
-Development of clones with control (inactive) ribozymes

CONCLUSIONS

Work in year one has led to the successful development of cell line harboring the c-Met ribozyme, essential for the ability to determine the effects of c-Met signaling on metastatic potential and uPA expression. The uPa inhibitors examined indicate that we can successfully inhibit signaling through the uPAR receptor. Thus, we are poised to assess the biologic effects of these pathways as described in task 2. We have also suggested that an alternative strategy, inhibition of the pathway by expression of the tumor suppressor gene PTEN/MMAC may be useful in completing task 2. As yet, the "so what" question cannot be answered, as the studies to assess the biologic effects of inhibition of the pathway are just beginning. Nevertheless, we have made very substantial progress on task 2, despite only beginning work on the project about four months ago.

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